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13. ABSTRACT *(Maximum 200*

We have continued to screen for BRCA2 mutations in our high-risk breast cancer kindreds and now have identified 10 deleterious mutations, 5 missense mutations of unknown significance and 3 polymorphisms. Our collaborator has screened an additional 150 families and identified 8 deleterious mutations and 1 missense mutation of unknown significance. There are an additional five families which are clearly linked to BRCA2 for which no mutations have been identified. We have begun to examine cases in those families for large deletions and/or rearrangements by probing Southern blots with 14 probes spaced within BRCA2. As we identify mutations within families, we expand the families to identify all mutation carriers in order to more accurately define age-specific penetrance and risks of other cancers. We have now collected 150 female and 91 male BRCA2 mutation carriers. In a collaborative study with the Breast Cancer Linkage Consortium, estimates are that penetrance by age 70 is 0.84 for breast cancer. We are just completing a collaborative study examining five recurring BRCA2 mutations to determine if there are founder effects and if so, the putative age of the mutations. We also are generating a mutation/haplotype database as collaborators send samples from families with mutations.

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Susan L. Neuhausen

PI - Signature Date

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DAMD17-94-J-4260

NOTE: PAGES 8, 9 AND 10 CONTAIN PROPRIETARY DATA

Annual Progress Report
Grant DAMD17-94-J-4260
Period: August 22, 1996-August 21, 1997

Introduction

Hereditary breast cancer is believed to account for up to 10% of breast cancer. We isolated BRCA1 in 1994. We and our collaborator on this grant localized BRCA2 in 1994, and our collaborator isolated BRCA2 in 1995, thus accomplishing the initial goal of this grant. The current aims are to characterize BRCA2. Characterization involves identification of mutations within families, analysis of those mutations in the families to identify all mutation carriers, and examination of age-specific penetrance for breast cancer, as well as risks of other cancers. Examination of founder mutations within populations such as the 999del5 in Icelanders and the 6174delT in Ashkenazi Jews may allow for better estimation of risk in these populations and directed genetic testing and counseling. BRCA1 and BRCA2 likely explain 80% of hereditary breast cancer based on linkage and mutation analyses, suggesting that there are other genes yet to be isolated which predispose to breast cancer. We still have families for which there is clear segregation of breast cancer within the family, yet no BRCA1 nor BRCA2 mutations have been identified. These families are being examined for linkage to putative candidate genes and regions in order to possibly localize BRCA3.

Body

Our aims for the previous year included 1) to identify BRCA2 mutations in high risk breast cancer families; 2) to estimate age and site-specific penetrance; and 3) to perform haplotype analysis to study the origin of the BRCA2 mutations. In April, 1997 we proposed to examine other putative breast cancer susceptibility genes and regions in families for which no BRCA1 or BRCA2 linkage or mutations were identified.

Progress in identifying BRCA2 mutations:

We have continued to look for mutations in our BRCA2-linked families. We have now identified a total of 10 families with deleterious mutations, 5 families with missense mutations of unknown significance, and two

polymorphisms. Five families with clear linkage to BRCA2 still have no identified mutation. No mutations were detected in an additional 22 families.

Our collaborator, Dr. Michael Stratton at the Imperial Cancer Fund in England, screened an additional 150 families for mutations in BRCA2 and identified nine mutations including one missense and eight frameshift mutations. One of the frameshift mutations was identified in a family with three male breast cancer cases. Not all of these families previously had been screened for linkage to BRCA2.

For the five families linked to BRCA2 for which no mutations have been identified by traditional sequencing of transcribed sequences and intron/exon boundaries, we are looking for evidence of large deletions which will have been missed by sequencing. Using published BRCA2 sequences in Genbank, we have constructed a restriction map of the entire BRCA2 gene. Using 5 restriction enzymes and 14 probes, we will probe Southern blots of DNA from early-onset breast cancer cases from each of these 5 families and look for evidence of large (> 1 kb) deletions and/or rearrangements. We are currently testing the probes to evaluate their restriction patterns individually and will then pool sets of 3 probes for the Southern blots of the familial samples. Depending on the results, we may also obtain new blood samples from cases in order to extract RNA and perform RT-PCR to look for deletions of individual exons.

We have continued to sample within our BRCA2 families and have now collected 91 male and 150 female BRCA2 mutation carriers, 77 of whom have female breast cancer, 7 with ovarian cancer, 10 with male breast cancer, 11 prostate cancer, and 24 with other cancers. The information on these mutation carriers will continue to be useful in examining penetrance and expressivity of BRCA2, as described in the next aim.

Progress in estimating penetrance and relative risks for BRCA2 mutation carriers:

In the last annual report, we had shown a table estimating penetrance of BRCA2 among female BRCA2 mutation carriers from 10 of our Utah kindreds. In a collaborative study with the Breast Cancer Linkage Consortium (BCLC), a mega-analysis of the genetic heterogeneity and penetrance for both BRCA1 and BRCA2 has been performed in a set of 237

families with at least 4 cases of breast cancer (Ford et al., submitted). Our data for all our high risk families, including BRCA1 and BRCA2 families, are included in this analysis. Risks of other cancers are still being analyzed. Overall, 52% of the families were linked to BRCA1, 32% to BRCA2, and 16% to other genes. The estimated cumulative risk of breast cancer was 28% (95% CI 9%-44%) by age 50 and 84% (95% CI 0%-47%) by age 70. Ovarian cancer risk was 27% (95% CI 0-47%) by age 70. We are currently collaborating on a second BCLC study examining the proportion of BRCA1 and BRCA2 mutations in families with site-specific breast cancer and less than 5 cases of breast cancer, stratified by the number of breast cancer cases per family.

Further examination of founder mutations:

During the last year, we have continued to examine founder mutations. In collaboration with researchers at the University of Iceland, we characterized the 999del5 mutation in Icelandic breast cancer families and found that 16/21 (76%) carried the 999del5 mutation and all carried the same haplotype (Thorlacius et al., 1996). We further explored the effects of the three recurrent BRCA1 and BRCA2 mutations in Ashkenazi Jews in a collaborative study examining families with two or more breast cancer cases with at least one of whom diagnosed at less than age 50 (Tonin et al., 1996). Among the 220 eligible families, 82 also contained cases of ovarian cancer. Twenty nine percent of the 138 families with only breast cancer had mutations in one of the three genes, and 73% of the 82 families with breast and ovarian cancer had mutations. In those families with only two cases of breast cancer and no ovarian cancer, 25% of families had a mutation. This suggests that if testing is to be offered, even small families should be eligible. We also looked at three other BRCA2 mutations which had been identified in individuals of Ashkenazi descent in our cohort of 130 Ashkenazi Jewish women with breast cancer, but none of them were observed.

Progress in performing haplotype analysis to:
1. Establish a database of mutations and haplotype.
2. Study the origin of BRCA2 mutations.

For this Aim, we put together a collaborative group of breast cancer researchers to contribute either samples or data from families with BRCA2 mutations. We are using 10 polymorphic markers spanning a distance of 6 cM around the BRCA2 gene to establish genotypes and subsequent haplotypes for each set of samples. Genotyping is by standard methodology. For each PCR reaction, we use 20 ng DNA in a 10 µl reaction amplified for 30 cycles of 95°C, 55°C, and 72°C, for 10 seconds each. The reactions are radiolabelled and then loaded onto 6% acrylamide gels. After 4 hours, the gels are dried and exposed to X-ray film. On every gel, there is a set of 5 controls with known genotypes. We can then assess uniform genotypes across gels. Haplotypes are assigned based on parent-child or sibling relationships.

We are establishing a haplotype/mutation database to be used as a resource to examine mutation haplotypes as a first step to identify BRCA2 mutations. Additionally, as researchers find other families which match haplotypes in this database, we will then identify other founder mutations. To date, we have analyzed DNA samples from 24 non-recurring BRCA2 mutations to establish genotypes. These samples were contributed from 8 different centers in North America and in Europe. We were only able to establish haplotypes for 3 of the mutations due to an absence of DNA samples from other family members. We are still collecting samples for this database and will include the haplotypes from the recurrent mutations discussed as the second part of this aim.

For the second part of this Aim, we examined recurring mutations in order to determine whether there was a common founder for each mutation, to estimate the age of the mutation, and to compare mutations to examine mutation specific phenotypes. Only those mutations for which there were five or more haplotypes could be analyzed. The 6174delT mutation which is frequent in Ashkenazi Jews (1.3%) was the most accurately estimated due to the large amount of data. In Table 1 are listed the mutations examined, the number of families examined, and the number of centers which contributed data. In total, we generated over 2,000 genotypes in order to examine the 7 cM region surrounding the BRCA2 gene.

Table 1. Number of families, centers, countries and haplotype or genotype

Mutation	FAMS	CTR	CNTRY	HAPLO	GENO
982 del 4	5	3	1	3	2
2034 ins A	5	3	3	3	2
3034 del 4	11	9	7	4	7
4484 del G	4	1	1	0	4
5573 ins A	3	1	1	3	0
6174 del T	69	12	7	22	47
6503 del TT	6	5	5	5	1
9254 del 5	3	2	2	3	0
9326 ins A	4	3	3	1	3
TOTAL	110	15	10	44	66

We calculated the number of generations (G) since the mutation originated, based on the same equations as in our examination of recurrent mutations in BRCA1 (Neuhausen et al., 1996). Each generation is approximated at 20 years. The age of the mutations for which we had 5 or more families are shown in Table 2. The confidence limits are large, however, the 3034 del4 is clearly a much older mutation than the others. The analyses are still being performed and a manuscript is in preparation. We are still analyzing the data to assess any phenotype/genotype correlations.

Table 2. Common Haplotypes and Likely Age of Origin of Mutation

Mutation	Core haplotype 260	1699	1698	171	1695	PWC[1]	1-LOD age interval
982 del 4	8	10	10	8	3	5/5	18 (4, 43)
2041 ins A	7	8	4	3	6	3/5	31 (12, 63)
3034 del 4	7	10	10	10	7	1/11	90 (52, 152)
6174 del T	8	10	12	4	4	5/69	29 (22, 38)
6503 del TT	6	8	8	10	7	3/5	45 (20, 89)

[1] Number of samples/families who are consistent with the core haplotype for all five markers

Progress in examining other putative breast cancer predisposing genes:

We have just begun to genotype 11 families comprising 122 DNA samples to analyze linkage for putative candidate regions and genes (Table 3). These 11 families were selected because their average expected LOD (ELOD) scores based on simulation analyses were all above 0.50 and therefore sufficient to detect linkage. The maximum ELODs for each kindred ranged from 1.1 to 3.2. Some of the candidate chromosomal regions to be analyzed for linkage include 8p, 11p15, 11q13 and 11q22, 15q14, 16q22-24, 18q11-13, 20q13, 22, and the candidate genes include the progesterone receptor, BCSC1, DAB2, e-cadherin, and PTEN/MMAC.

Table 3. Families being examined for other breast cancer susceptibility genes.

Kindred	Total cases	DNA from cases	Age range (yrs) diagnosis
1917	5	3	42-60
1929	7	3	33-72
2019	10	4	43-79
2036	5	3	34-60
2260	5	4	31-50
2262	6	5	48-53
2308	9	4	36-71
2324	6	3	38-81
2329	15	5	29-90
2370	3	3	39-64
2381	6	4	41-70

Conclusions:

During the previous year, we have further characterized mutations in BRCA2 and examined some founder mutations in detail. It is clear that individuals of Ashkenazi Jewish descent are at increased risk of breast cancer and that genetic testing can be stratified to first test for the three recurrent mutations which appear to account for approximately 20-30% of breast cancer in this population. However, there are other BRCA1 and BRCA2 mutations which have been identified in this population, so that a negative result for the recurrent mutations does not preclude another mutation. There are recurrent mutations in other populations which may be important for risk assessment including the 999del5 mutation in Icelanders, the 4484delG in the Swedes, and the 5573insA in the Dutch. From our collaboration with the BCLC, better estimates of age-specific penetrance are now available which can be used in assessing risks. Once the analysis of risks of other cancers is completed, those estimates will be included in clinical risk assessment.

For the next year of the project, our goals are to 1) identify mutations in BRCA2 which are due to rearrangements or large deletions; 2) examine tumors from familial breast cancer cases and obligate carriers to assess frequency of loss at BRCA2 vs. BRCA1; 3) continue to identify additional mutation carriers within our BRCA2 families for use in epidemiological studies of risk factors for BRCA2 mutation carriers; 4) continue to expand our haplotype database; and 5) screen our families with no BRCA1 or BRCA2 mutations for putative candidate loci for BRCA3.

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